# Yellow Disease of Sheep

(*Parasitic Ictero-Haematuria.*)

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## Montana Sheep Commission

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FIG. 1.

Fig. 1. Sheep 56. (four-year-old ewe.) Advanced case of ictero-haematuria, obtained May 18, 1903. Temperature at 1 P. M., 101.8°; at 8 P. M., 101.4°; at 8 A. M., May 20, 102.4°; at 3 P. M., 101.2°; at 7:45 P. M., 101.4°. Died May 22. Red corpuscles, 1,860,000 per cb. mm.; white corpuscles, 13,000 per cb. mm.

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# The "Yellow Disease"

(**Parasitic Ictero Haematuria**)

OF

## SHEEP IN WESTERN MONTANA.

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The following studies were undertaken at the suggestion of Dr. M. E. Knowles, State Veterinarian of Montana, to whom I am indebted for much valuable assistance and advice in carrying on the work. A laboratory fully equipped for pathological and bacterological work was established in Helena, and a field laboratory at Race Track, in the Deer Lodge Valley.

The results in brief, are these:—

1. A disease is endemic among the sheep of the Deer Lodge and Silver Bow valleys, known to pathologists as parasitic ictero-haematuria, and locally as the "yellow disease," "yellow hide," or "red water." It is without doubt identical with the "carceag" of Roumania, described by Babes in 1892, and three years later by Bonome in Northern Italy.

2. As nearly as can be learned, the disease first appeared among a band of Merinos in the winter of 1890, near Race Track, ten miles south of Deer Lodge. It soon spread to flocks in the vicinity, and during the next four or five years caused such severe losses as to drive several sheep-raisers out of the business.

3. Beyond the Valley the disease has spread more slowly. In 1892 or 1893 it first appeared among the sheep of T. Cowles Miles, Esq. near Silver Bow. During the past winter it has extended as far south as Freely, on Divide Creek, sixteen miles south of Butte. In whatever locality it appeared, it continued during the subsequent years.

4. The first cases develop in late fall or early winter. Fresh cases appear at intervals throughout the winter and spring, being especially numerous among ewes before and after lambing. No cases appear after July 1, and very few or none in June.

5. The disease is rapid in its course, and probably always fatal.

6. It is most prevalent among sheep that feed on the bottom

lands. Flocks within the infected area, kept on high ground, generously supplied with good feed and pure water, sometimes escape entirely.

7. Although cases develop after the sheep are taken to the hills in the spring, it is probable that they have contracted the disease in the low grounds, and it has remained latent for a period.

8. Three-and-four-year-old ewes are the most susceptible, then wethers of the same age. Lambs and bucks are immune.

9. Sheep brought into the infected area usually remain immune throughout the first year, but during the second winter the disease appears among them.

10. Sheep taken from the infected area to other parts of the State soon cease to die of the disease, and do not transmit it to other sheep.

11. A minute parasite (Piroplasma ovis) has been found in the blood corpuscles of every case. A very similar parasite (Piroplasma bigeminum) occurs in the corpuscles of southern cattle, and is known to cause Texas Fever.

12. The causation of ictero-haematuria by the blood parasite has not been experimentally demonstrated, either here or abroad. Experimental inoculations of the blood of diseased sheep into the veins or under the skin of healthy sheep have uniformly failed to reproduce the disease. Ticks from sheep dying or recently dead of the disease, placed on healthy ewes and wethers, likewise proved innocuous.

13. The disease is characterised by a general icterus or jaundice, the whole body becoming more or less yellow. There may or may not be edematous areas under the skin. The internal organs most affected are the liver, spleen and kidneys. In extreme cases, especially pregnant ewes, there is marked haemorrhagic edema of the peritoneum, bladder, etc., and great enlargement of all lymph nodules. The blood is greatly impoverished and anemic, the red corpuscles falling as low as one or two millions per cubic millimeter. The urine is coffee-colored, owing to the presence of blood. The kidneys are dark, almost black, and intensely congested. Owing to the clogging of the bile-capillaries with thickened bile, the liver presents a yellow hue, sometimes a mottled aspect, and is extremely friable, at length becoming necrotic. The gall-bladder is often greatly distended and the bile is sometimes very thick with mucus in advanced cases. The spleen is always enlarged and the pulp is very dark brown, almost black. Not infrequently there are ecchymoses in the heart muscle, especially of the left ventricle.

## Historical.

This disease was first described by Babes, Bucharest, Roumania, in 1892. It is endemic in the low islands and delta of the Danube, ap-

pearing every year among the immense flocks driven there from the hills, and is most prevalent in May and June. It is known to the Roumanians as "carceag." In 1893 and 1894 Bonome studied eight cases in Northern Italy, and although he described the disease as distinct from "carceag" there is no doubt that it is the same. The name given by Bonome, "Parasitic Ictero-haematuria" is admirably descriptive, and has been retained. Both Babes and Bonome found a minute parasite in the blood corpuscles which they regarded as the cause of the disease. The position of these parasites in the organic scale was not correctly determined by either Babes or Bonome. The former regarded it as intermediate between the Bacteria and the Protozoa, thus making it a group by itself (Haematococcus) in which he placed also the very similar parasite of Texas fever or haemoglobinuria of cattle. Bonome, in view of the active amoeboid movement of the organism was inclined to group it with the Amoebae, and the endogenous, spore-like mode of reproduction led him to establish the special group Amoebosporidia. Bonome did not regard the parasite he had studied as identical with that found by Babes, which he speaks of as "a parasite of vegetable origin, capable like all bacteria of growing on artificial media." While Babes claims to have obtained a growth of the parasite of haemoglobinuria on blood serum containing haemoglobin, no one else has succeeded in doing so, either with this parasite or any other of its class.

Starcovici, a disciple of Babes, while contributing little that is new to the subject, has drawn an interesting and valuable comparison between haemogloburia, Texas fever and carceag or icterohaematuria. He regards them as three distinct though closely related diseases,—a view not shared by pathologists of today as regards haemoglobinuria and Texas fever. He follows Babes in regard to the systematic position of the parasites as intermediate between the Bacteria and the Protozoa, but arranges them under two genera,—Pyrosoma, including only the parasite of Texas fever, named Pyrosoma bigeminum by Theobald Smith, and the new genus Babesia, including two species, B. ovis, the parasite of carceag, and B. bovis, the parasite of haemoglobinuria. Starcovici does not define his new genus and there are no sufficient grounds for retaining it. The three species established by Starcovici have been reduced to two, as the haemoglobinuria of Europe and the Texas fever of America are now regarded as the same disease. Hence, in the absence of morphological differences in the parasite there is no reason to think that we have to do with more than one species. As the generic name Pyrosoma had already been given to a genus of Tunicates, it was changed to Piroplasma by Lebbe in 1899. Several new species have been discovered in recent years, each the cause of a specific blood disease in one of the higher Mammalia. Thus we have canine piroplasmosis produced by Piroplasma canis, ictero-haematuria of sheep, with P. ovis as its

prime, if not only, causative factor, P. bigeminum of bovine haemoglobinuria, and most interesting of all from the standpoint of human medicine, the so-called "spotted" or "tick fever" of the Rocky Mountain Region has been brought into this category of diseases through the discovery by Wilson and Chowing of a specific blood parasite, Piroplasma hominis. This group of parasites is now regarded as belonging to the Haematzoa, and as closely akin to the various malarial organisms. They are smaller than the malarial parasites,—some of them much smaller—and of simpler structure. Their life-history is almost entirely unknown. The brilliant researches of Smith and Kilborne have demonstrated beyond a doubt that the Texas fever organism is transmitted by female cattle ticks which take the parasite into their stomachs with the blood they suck. Of the changes it undergoes in the tick we have no knowledge whatever; but we do know that in some way it passes into the ova so that the young ticks become infected. These in turn infect the cattle they fasten upon. Piroplasmosis of the dog as it occurs in Cape Colony has also been shown by Lounsbury to be transmitted by a species of tick, Haemaphysalis Cachi (Andowin). The same disease among the dogs of France is considered by Nocard and Motas in their valuable contribution to the subject (1902) as very probably transmitted by another tick Dermacentor Uticulatus. It is of interest to find that the tick held responsible for the transmission of the "Spotted fever" of the Rocky Mountains is regarded by Stiles as either this species or a Dermacentor closely allied to it.

As regards the transmission of Piroplasma ovis from sheep to sheep we know nothing. Although nearly all sheep are infected by the so-called "sheep tick", Melophagus Orinus, this is no true tick but a wingless fly; therefore an insect whereas the true ticks are eight-legged and more nearly allied to the spiders.

In 1895 appeared the first scientific account of the ictero-haematuria in Montana, by Dr. W. L. Williams, then Experiment Station Veterinarian at Bozeman. Before that the disease had been recognized as early as 1892 by the State Veterinarian, (Dr. H. Holloway) but erroneously diagnosed as anthrax. According to the statement of Mr. T. Clowes Miles of Silver Bow, the "yellow disease" first appeared in the Deer Lodge valley in 1890 in a band of Merino ewes owned by Mr. N. Bielenberg. The epizootic was so severe that by spring over two-thirds of the band had perished. It is possible that these Merinos were imported and brought the disease with them; but this I have not been able to verify. In view of the fact that sheep had been in the valley since 1875 the tardy appearance of the disease, if indeed it is endemic, is remarkable.

According to Williams the disease attained great virulence the following year, when it ravaged four or five large bands. At that time there were more sheep in the valley than at present, and the losses

were correspondingly greater. The loss from the yellow disease alone, according to Williams, was 1600 per annum. In fact, it proved so destructive several large sheep owners were driven out of business.

Williams was the first to identify correctly the Montana disease as identical with the "carceag" of Roumania and the ictero-haematuria of northern Italy. There are slight differences, it is true, in the clinical features, but there are so many points of resemblance there can be no reasonable doubt regarding the identity of the Montana and the European disease. Williams gives a full account of the symptoms and clinical picture, and determined the presence of the parasite in the red corpuscles.

## Distribution of the Yellow Disease.

In view of the fact that sheep are constantly being driven out of the Valley to other parts of the State, it is remarkable that the disease has not spread more rapidly and widely. Its extreme limits during the past winter have been Feely, sixteen miles south of Butte, and a point about three miles west of Deer Lodge. These points are about 50 miles apart. Williams in 1895 estimated the infected area at 300 square miles, and it is certainly not much greater today.

All sheep owners with whom I have conversed agree that the disease does not occur until the sheep are brought in from the summer range in the hills in late fall or early winter. It then usually appears with great promptitude, but is very irregular in its incidence. A few sheep, or perhaps only one or two are attacked, then there will be no further cases for weeks or even months. It is reputed to increase with the advent of warm weather, but this I was not able to verify. It is especially prevalent among ewes after lambing, doubtless because the system is then greatly weakened. No cases develop after the first of July and during the past spring I saw none after the first week in June.

The occurrence of the disease among the sheep of the valley is remarkably irregular. While some owners met with considerable losses, others in the same vicinity, perhaps only a mile or two away, would lose very few or even none from this disease. There was a notable difference, however, in the way in which the flocks were kept. Those allowed to range over the bottom lands, to drink from the river polluted with smelter tailings, and fed with native hay cut from the bottoms, (which are often flooded in spring) suffered the greatest losses; while sheep kept on higher ground away from the river and fed alfalfa, supplied with pure water and salted frequently, often escaped entirely. It is commonly believed by the sheep raisers that sheep brought into the valley are immune

during the first winter, but during subsequent years are peculiarly susceptible. This belief is apparently well-founded, but obviously a single year's observation has not been sufficient to verify it.

## Report of Cases.

During the winter and spring about thirty cases came to my personal knowledge, but the number that actually occurred must have been much greater. It proved impossible to hear of every outbreak, on account of the very considerable area over which the disease has spread, the sparsely settled character of the country, and above all on account of the marked reluctance on the part of some of the largest sheep owners to assist the investigation in any way whatsoever. Twenty-two autopsies of sheep affected with this disease were made, and portions of the organs most affected were preserved for microscopic study. A great number of blood smears were taken from all fresh cases, and numerous blood-counts were made. The urine of a few cases was analysed. In a few instances the entire liver, spleen and kidney were preserved in their natural color and condition in Kaiserling's fluid.

The first case occurred within three miles of Warm Springs, November 25, 1902. Although no autopsy of this case was made, and the characteristic jaundice was not apparent externally, blood smears showed numerous parasites in the red corpuscles. It was subsequently learned that the general icterus is not an absolutely constant feature. It varies from a slight buffy tint to deep saffron, and in one case seen in January regarding which there was not the slightest doubt it was not perceptible either externally or internally.

The next cases seen developed in the flock of Mr. T. C. Miles, in the Silver Bow valley, about December 15. The disease appeared immediately upon the bringing in of the sheep from the range. There were only two cases, both typical and well-marked, and it is remarkable that these were the only cases in this band of 800 during the entire winter and spring.

The next report of the disease came from Mr. W. J. Farmer, two miles south of Feely. In the middle of January four cases occurred in his band of 1,800; three were dead when I arrived, on the 12th, and another case, a four-year-old ewe, was discovered the following morning, and died soon after. The temperature less than an hour before death was 102.6 and the respirations, which were very labored, were 27 per minute. As often happens in this disease, the animal died in convulsions. A thorough autopsy was made, and the usual clinical picture was presented. Unlike the two cases examined at Miles', and the three other cases at Farmer's, there were haemorrhagic edematous areas under the skin; this is not a con-

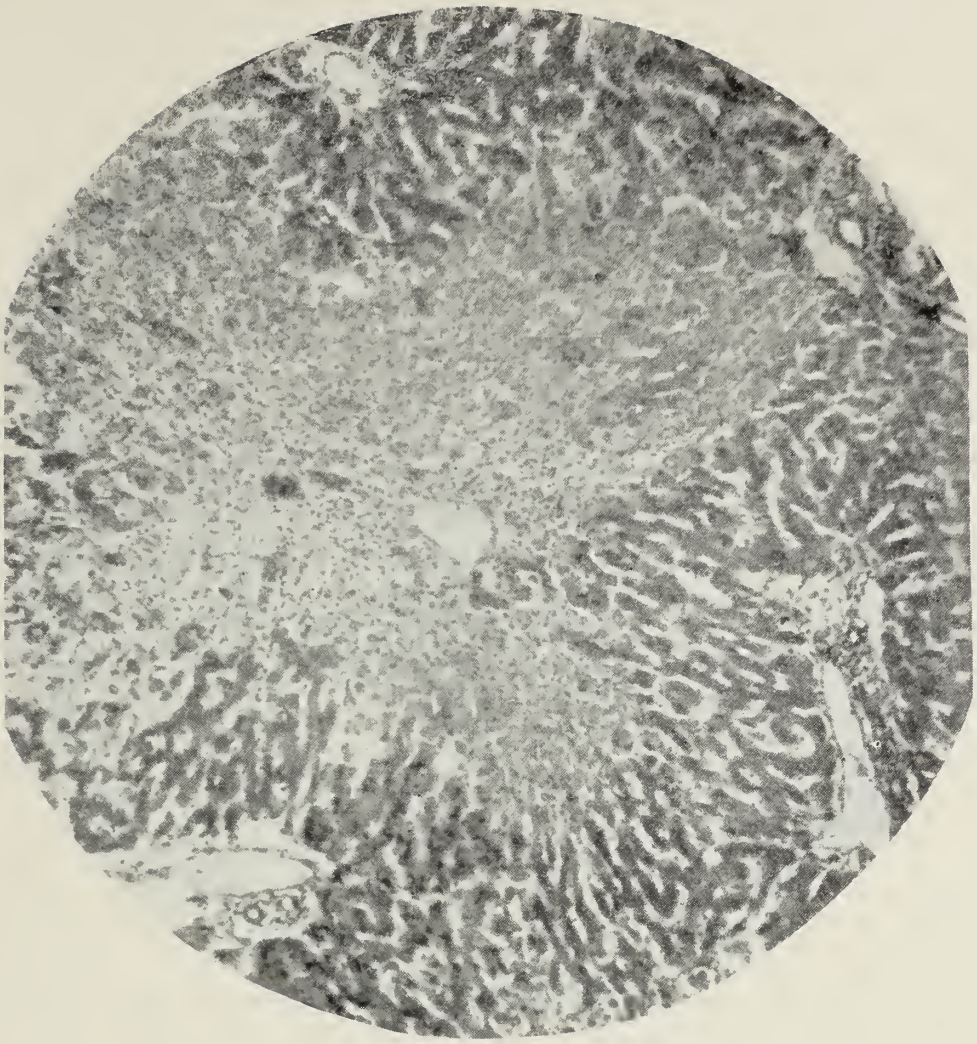

FIG. 2.

Fig. 2. Photo-micrograph of section of liver of Sheep 62, showing one entire lobule with its central vein surrounded by extensive necrosis of the liver cells. The trabecular arrangement of the parenchyma is very distinct, owing to the great distension of the bile-capillaries. X 125.

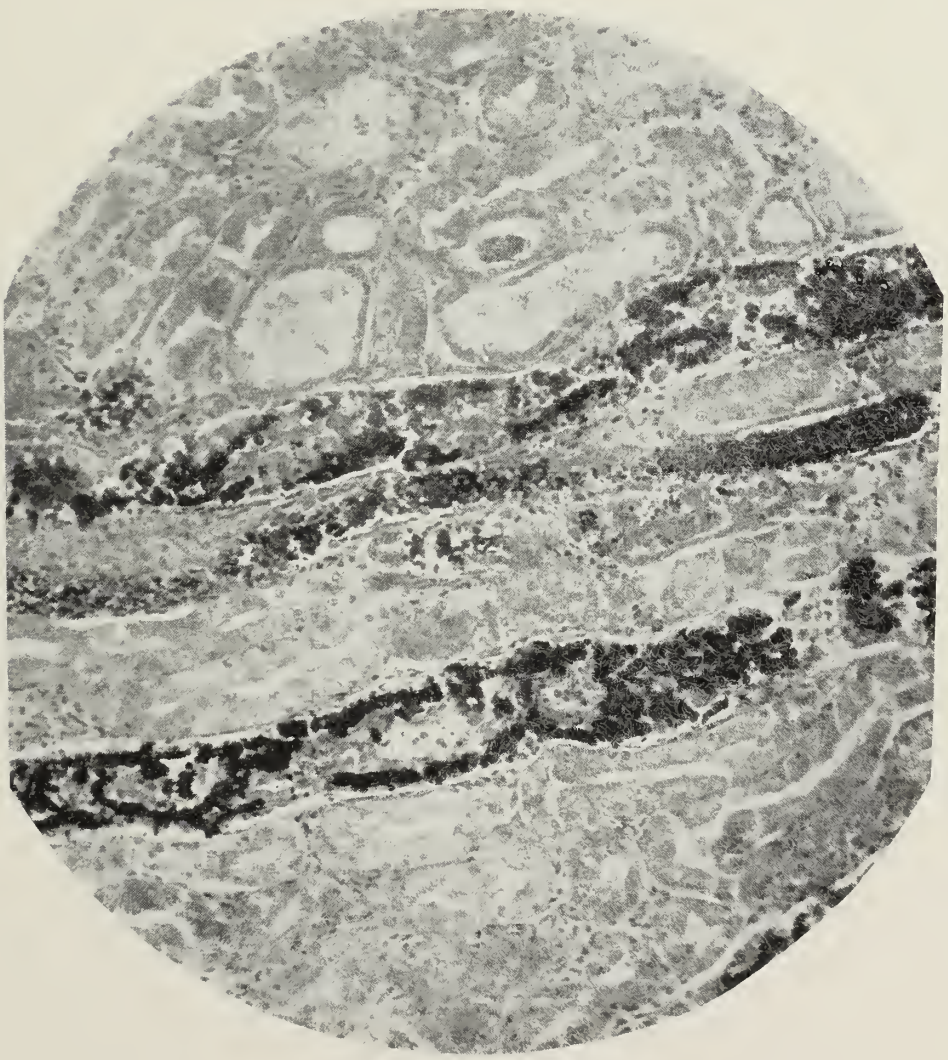

FIG. 3.

Fig. 3. Photo-micrograph of section of cortex of kidney of sheep 15, showing three tobules with strongly-pigmented epithelium.

stant feature. The small quantity of urine in the bladder was dark coffee-color, and microscopical examination revealed numerous red corpuscles. These however would not account entirely for the very dark color of the urine, which is unquestionably due largely to the presence of altered haemoglobin excreted by the kidneys. The spleen was not greatly enlarged, but the pulp was very dark. The kidneys were greatly congested, bluish before removing the capsule, which peeled off very readily. Within, the cortex and medulla were the same color, almost black, and practically indistinguishable to the naked eye. The liver was not necrotic and the bile stasis not marked. The gall-bladder was not greatly distended. The heart was free from ecchymoses, the lungs normal. The large intestine and rectum were opened, but no lesions in the folds such as Babes describes were found. The blood was pale and watery, and there was marked leucocytosis. Parasites were found in a small percentage of the corpuscles. As not infrequently happens, these were not detected in the fresh blood but only in stained preparations.

The next case observed was a two-year-old ewe belonging to Hempstead and Boyle, near Race Track, in the Deer Lodge valley. This was seen February 2. I was informed by the owners that this ewe had been sick for seven days, during which time it had eaten little or nothing. The animal was extremely emaciated, and so weak it could hardly stand. Neither the skin nor the white of the eye were perceptibly yellow, and after killing the animal the usual icteric condition of the carcass and internal organs (except the liver) was imperceptible. There was no doubt concerning the identity of the disease, as shown by the condition of the liver, spleen, and kidneys. Smears from the spleen showed also the presence of the parasite in the corpuscles.

In the second week in February came the report of further losses near Race Track, in the band of Mr. Hugh Magone. Only one or two cases of the yellow disease were known to develop in this band of 1,000 ewes and bucks, which, though allowed to graze on the river bottoms were given excellent care and had pure water to drink. Arriving at Race Track February 10, I soon determined to make this my field headquarters, as the place is within easy reach of nearly all the sheep now kept in the valley. Mr. Ed. Calvert furnished me laboratory accommodations, and I had the use of his team and personal services during the rest of the season.

The greater number of cases developed in the two bands (2,100 ewes and 2,100 wethers) of Hempstead and Boyle and in a band of 2,300 ewes and wethers owned by W. M. Montgomery and Co. of Anaconda, and kept on the ranch of Mr. Beckstead, three miles south of Race Track. The losses in this band were so large that the owners moved them to Dillon about the middle of March. Up to that time five cases of ictero-haematuria had to my knowl-

edge appeared in this band, and in all probability the actual number was at least twice as great. During the winter and spring I personally examined thirteen cases in the two bands of Hempstead and Boyle. There is no question that ictero-haematuria causes the greatest number of losses of any one sheep disease in the Deer Lodge and Silver Bow valleys. Two other diseases, croupous pneumonia and the "big head" caused greater losses in May and June, but they prevail for a much shorter period. The former in fact lasted for only a few days during a cold wet snow storm in May, in the midst of lambing.

The last cases of the season were three seen on June 5 in the ewe band of Mr. Eli Desorde. This band had been snowbound in Cottonwood Basin during the severe storm of the latter part of May, during and after which the losses had been quite heavy. During the entire winter and early spring not a single case, so far as known, occurred in Desorde's band. During and after lambing the losses were heavy in all the bands, and fully half were from ictero-haematuria.

## Symptoms and Gross Pathology.

Probably no case was seen in its earliest stage. A sheep in a large band is practically lost, and nothing is recognized to be the matter with it until the disease is far advanced. Among the first indications of ictero-haematuria are loss of appetite, indifference, and a tendency to keep apart from the flock. When the band is being driven these sheep are invariably in the rear. They either stand dumpishly when left to themselves, or wander aimlessly about. The head is drooped and there is a marked sagging of the loins (see Plate I). If pursued they run in an uncertain, wavering manner, and turn aside for neither water nor fences. The animal urinates frequently, apparently with some difficulty, and the urine is very dark with blood and bile pigments. The feces are in large irregular nodules, sometimes covered with mucus; rarely there is diarrhoea. The animal is weak and trembling, and the extremities become cold some time before death. The temperature is usually subnormal, especially in the advanced cases. Once, in case of a pregnant ewe, a temperature of 98.5 was recorded, and temperatures between 102 and 101 are very common. In only one instance was a high temperature (104.6 degrees) obtained. This was surprising, inasmuch as ictero-haematuria has been considered a fever-disease, like Texas fever, in which the rise of temperature is very notable. Williams does not record any temperatures, but says "a moderate fever is present." Bonome's records, however, agree with mine. He states that the highest temperature obtained by him was 39 degress C. (102.4 degrees F.). Starcovici states there is

high fever, with temperature of 40 to 42 degrees C. (104 to 107.6 degrees F.). It was obviously important to ascertain accurately what may be considered the normal temperature of healthy sheep in the same locality and at the same time of year. A temperature-record of eight sheep was kept from March 3 to March 28, being taken either once or twice a day. Some of these sheep had been inoculated with blood from cases of ictero-haematuria, and a rise of temperature was anticipated, but in no instance did it occur in a decisive manner, indicating the inception of the disease, and no case of ictero-haematuria developed among the experimental sheep. What may be regarded as the normal temperature of sheep is highly variable, and in the same animal, in apparent good health, may vary in 24 hours from 102.8 degrees to 104.2 degrees, as shown by the record of No. 29 (a wether) on March 13 and 14. Probably a fair average for sheep in winter and spring is 103 degrees. It is thus seen that the temperature of 104.6 degrees recorded for an icteric sheep is not beyond the range of normal temperature. In no other instance was it so high as this.

Only adult ewes and wethers are susceptible; lambs and bucks seem to be wholly immune. The greater number of cases that came under my observation were two-year-olds and over, and more ewes than wethers. The ewe seems susceptible during and after pregnancy, when the system is weakened. The animals attacked are almost always fat and apparently in excellent condition, and they generally die too soon to lose much flesh. It is almost always useless to look for a case of the yellow disease in a lean and miserable-looking "hospital band."

The gross lesions implicate principally the organs concerned in the elaboration and the purification of the blood. The liver, spleen, and kidneys are the most affected, but in extreme cases all the lymph nodules are enlarged, and there are hepatised areas in the lungs. It is quite possible, however, that the latter are of independent origin, and have nothing to do with the disease. Extravasations of blood in the myocardium, especially of the left ventricle, are very frequent, but not a constant feature; the same may be said of the haemorrhagic edemas in the subcutaneous tissue of the neck and brisket. In the three cases of pregnant ewes examined there were extensive ecchymoses and edamatous areas within the body cavity, implicating chiefly the connective tissue of the peritoneum, the mesentery, the omentum and especially the bladder, the walls of which were a centimeter thick.

Analysis of the urine frequently showed a very high percentage of albumen, too much in fact to measure without dilution by the scale on Esbach's tube. In other cases it was from 6 to 9%. In one case (sheep 35), where the urine was not red or dark, and appeared

almost normal, the amount of albumen was only 1.5%. The urine is neutral or alkaline. For sheep 33 a specific gravity of 1.015 was found.

The condition of the blood is very diagnostic. If it appears rich and bright red to the naked eye, it is a sure indication that the disease is in an early stage. Even then the kidneys and spleen may exhibit the characteristic congestion and dark color. All advanced cases have the blood very thin, watery, and dark colored. While the normal number of red corpuscles at the altitude of Race Track (4,700 feet) is from 13 to 14 millions, icteric blood-counts range from 1 to 3 millions per cubic millimeter. In only one instance was the count as high as 9 millions. The number of white corpuscles is markedly increased, and counts vary in different instances from 7,000 to 28,000 per cubic millimeter.

## Minute Pathology.

Small pieces of the liver, spleen, kidney and a few of the other organs were fixed in Zenker's fluid, Orth's fluid, or in 98 per cent. synthol, imbedded in paraffine by the chloroform-infiltiation method, and cut in sections five to ten microns thick. Several of the standard staining methods were employed,—haematoxylin-eosin, polychrome methylen-blue and eosin, haematoxylin-picro-acid-fuchsin, Heidenbrain's iron-alum-haematoxylin, and Apathy's chrome-alum-haematoxylin. The two first mentioned and the iron haematoxylin gave the most satisfactory results.

The picture which the liver sometimes presents is very striking. Externally, it is beautifully mottled in a sort of vermiculated pattern. This was specially notable in the liver of Sheep 62, a large four-year-old ewe. The mottled effect obtains throughout the liver, appearing in every section. Examination at low magnification shows that the center of every lobule is necrotic. It is composed of the debris of cells that have undergone degeneration (See Fig... Plate II). The central vein is still intact. The radical bile capillaries are greatly distended, so that the liver cells thus left in widely separated columns, exhibit their trabecular arrangement very plainly. Very little blood flows from the liver on cutting it; this is in accordance with the general impoverishment of the blood. No case so extreme that any considerable portion of the liver had become completely necrossed was seen by me, but such undoubtedly occur. This condition is unquestionably brought about by the gradual extension of the central necrosis until it involves the entire lobule, and this occurring in many adjacent lobules an extensive necrosis is the result. The central necrosis is itself a late condition not seen in the majority of cases.

The spleen, as already stated, becomes greatly enlarged, this

enlargement being due to its turgescence with blood. Probably there is little or no increase of the spleen pulp.

Weights of icteric spleens, taken soon after death, are as follows:

Sheep 55 (ewe), weight 81 pounds, spleen 129.6 grains.

Sheep 60 (ewe), weight 77 pounds, spleen 292.84 grains.

Sheep 62 (ewe), weight 92 pounds, spleen 227.14 grains.

The normal weight, as shown by wethers 28 and 29, is 45 to 60 grains.

Phagocytes containing the remains of red corpuscles are exceedingly abundant. The later stages of the resorption of the corpuscles are indicated by the occurrence of groups of coarse amorphous masses of globules of haemosidarin, a derivative of the haemoglobin of the ingested corpuscles. This substance appears as refractive, grass-green granules in smears of the fresh spleen. In material fixed in Zenker's fluid the granules have a deep yellowish tint, and do not stain with either acid or basid dyes.

The kidneys are also extremely turgid. The following table gives a fair idea of the extent of the enlargement:

Sheep 55, weight 81 pounds, right kidney 110 grains.

Sheep 60, weight 77 pounds, kidney 114.4 grains.

Sheep 62, weight 92 pounds, right kidney 131.4 grains.

Normal sheep of about the same weight (Nos. 28 and 29) were found to have a kidney-weight of 60 grains.

The kidneys exhibit both macro and micro-scopically an intense nephritis. As all the material at hand is from advanced cases, it is impossible to state positively what pathological changes first occur, but there is reason to think it begins as a cloudy swelling and degeneration of the epithelial lining of the tubules. The cytoplasin of these soon becomes crowded with fine, then with coarse, dark brown pigment (see Fig. 3, Plate II). This pigment eventually obscures the cell-structure. Not all tubules are thus affected, but in some it extends from the outer edge of the cortex far into the medulla. In a lightly stained preparation these pigmented tubules stand out very prominently. Many tubules have tube-casts, the material of the cast being either albuminous or composed of red corpuscles in more or less advanced stages of disintegration. They still stain intensely with eosin, and are thus distinguishable from the albuminous coagulum. That a portion of this blood at any rate passes down with the urine is shown by the fact that corpuscles are found in urine taken from the bladder.

### The Parasite, Piroplasma Ovis.

In many of the red corpuscles of the fresh blood a minute, highly refractive body may be seen under high magnification. This is the germ or parasite which is the prime cause, in all probability, of the

disease ictero-haematuria. It has not been seen with certainty outside of the red corpuscles, for certain similar bodies seen floating in the blood plasma have not been positively identified as the parasite. Neither have I been able to detect it in the spleen-pulp cells, nor in the tubular epithelum of the kidneys, as described and figured by Bonome. In view of the high degree of specialization of related parasites, notably those of Texas fever and malaria, it is improbable that piroplasma ovis infects any cells of the host except the red corpuscles. It is true that some of the epithelial cells of the renal tubules contain a stainable rounded body of about the size and aspect of the intarcorpuscular parasite as ordinarily seen after staining; but these are no safe grounds for homologising it with the latter.

Several of the standard blood-stains were employed,—Nocht's modification of Romanowsky's method, Wright's modification of Jenner's, Goldhorn's stain, and the carbolized polychrome methylen-blue recommended by Nicolle and Abdil-Bey for the staining of haemoglobinvaria blood-smears. The special stain employed with such happy results by Nocard and Motas in their classic work on the piroplasmosis of the dog was tried, but without success—possibly because Hochst's eosin was not obtainable. On the whole the most instructive preparations were those stained by Nicolle and Abdil-Bey's method, and these were the only ones that brought out the cell-body of the parasite, which is invisible not only in the living condition, but also after staining by nearly all methods. The minute refractive body from 5 to 3 microns in diameter, which is seen in fresh blood and stains intensely with basic dyes, is undoubtedly either the nucleus or the caryosome.

Not over 10 per cent of the red corpuscles have been found to be infected in any instance, and usually less than one per cent. Possibly in some portion of the body a higher percentage of corpuscles are infected, as has been found in malaria and Texas fever; such a place, however, has not been found, although the blood of the red marrow, spleen, liver, kidney, and heart-muscle has been carefully searched.

In all cases of ictero-haematuria in which the blood has been examined there is notable variation in the size of the corpuscles. (See Figs. 4 and 5, Plate III). While the normal red corpuscles of sheep measure 5 microns in diameter and are very uniform in size, the corpuscles of icteric blood vary from 2.6 to 7 micronus in diameter. This poikilocytosis has been noted in Texas fever by Smith and Kilborne after the red corpuscles had fallen to 3,000,000 per cb m m., and in piroplasmosis of the dog by Nocard and Motas.

The presence of basophilic granules in the red corpuscles was noted only in sheep 13 (See Fig. 5, Plate III). Smith and Kilborne found such corpuscles constantly in advanced cases of Texas

fever, and also in a sheep and a cow after severe bleeding. As they occur in human blood in pernicious anaemia, lenkaemia, malaria, etc., it is evident that they are in some way associated with the impoverishment of the blood. Their frequent absence in icterohaematuria is at present inexplicable.

Normoblasts (nucleated red corpuscles) are occasionally seen, especially in spleen pulp smears, but never in striking numbers. There is on the whole very little to indicate that the used-up corpuscles are being rapidly replaced by new ones. In order to detect this by blood-counts it is of course necessary to have a complete haemal history of a recovery-case, and no one has yet had the opportunity to study such a case.

The stainable portion of the parasite is usually of spherical form, and either single or double (Fig. 4, Plate III). It stains intensely with basic dyes, and varies in size from a mere speck to a body .. microns in diameter. The well-known paired piriform stage of the Texas fever organism has not been detected. The form of the parasite as brought out by staining with Nicolle and Abdil-Bey's carbolised methylen-blue occasionally suggests amoeboid changes of form (Fig 5, Plate III), such as the malarial organism exhibits. Such changes, however, have not been seen in the fresh blood, although I had no difficulty in making them out in the freshly-drawn blood of a tick fever case in the Bitter Root valley. It should be stated, however, that in the latter instance a Zeiss apochromatic 2 m m. oil-immersion was employed, whereas in all observations on fresh sheep's blood the best lens available was a Bausch & Lamb 1-12. Furthermore, suitable warm-stage appliances for such delicate observations were not at hand.

Regarding reproductive phases of the parasite there is very little to record. The presence of two in the same corpuscle is by no means infrequent (Fig 5, Plate III), and the position of these is often such as to lead inevitably to the conclusion that they are the offspring of a single organism. In one case four were seen to form a close group in the corpuscle.

To how great an extent the piroplasma is responsible for the immense loss of corpuscles in the circulation it is impossible to say, because corpuscles are constantly being lost with the urine, being ingested by the phagocytes in the spleen, and being stored up in every haemorrhagic area and congested organ of the body. There is every reason to believe that the destructive effect of this organism is not purely mechanical, although of course it is possible that they prey upon the red corpuscles to such an extent as to reduce their numbers very considerably. There is little doubt that they also secrete a toxin as virulent as that of the pathogenic bacteria, and that this is the most important cause of their deadly effect.

The pathway of invasion by the piroplasma is still unknown.

Judging from analogy, it is some biting insect, tick, or other ecto-parasite of the sheep, which injects the organism directly into the blood. Suspicion naturally attaches to that constant pest, the so-called "sheep-tick," Melophagus ovicrus, but thus far all experiments to inoculate healthy sheep by placing upon them ticks from those affected with ictero-haematuria have failed. Bonome's hypothesis that it enters by the alimentary tract seems very improbable. Such a mode of entry has not been determined for any of the blood-parasites.

The interesting and important fact has recently been established by Wilson and Chowning (1902) that another local disease in Montana, the "spotted fever" (more appropriately, as suggested by Anderson, tick fever) of the Rocky mountain region, is caused by a blood-parasite (piroplasma hominis, Wilson) so similar to P. ovis that it cannot be distinguished by microscopical inspection, even with the most powerful lenses. Occasionally, however, it has the twinned form of P. Vigenimum. (See Anderson, Pl. II, Fig. 4).

## Experiments.

The practical impossibility of getting spontaneous cases in the early stages in order to watch them through their course rendered it highly important to be able to produce the disease at will by inoculating healthy sheep with the infected blood of icteric sheep. Successful inoculations would moreover dispose once and for all of any doubts concerning the causation of the disease by the parasite. Such experiments hitherto have always failed, for some reason not understood. The failure in case of ictero-haematuria is the more remarkable, because the kindred diseases, Texas fever and the piroplasmosis of dogs are easily transmissible in this way. Bonome has expressed the opinion that not alone the parasite but some derangement, probably of the digestive system, is necessary to produce the disease. In other words, a perfectly healthy animal will not contract ictero-haematuria, even though the virulent germs be injected directly into the circulation. From my own experiments I am convinced that this view is correct. Eight sheep (three ewes, four wethers and one imperfectly-developed buck) received injection of blood from advanced cases of ictero-haematuria. The blood was generally injected directly into the jugular, but also subcutaneously, and in quantities varying from one to twelve cubic centimeters. In one instance group-up spleen pulp was used for a subcutaneous injection. The blood was taken perfectly fresh from the jugular of an icteric sheep in the last stages of the disease. It was sometimes defibrinated, sometimes injected directly. The sheep were kept by themselves, well watched and cared for, and careful record of temperature was kept, as it was anticipated there would be fever as a

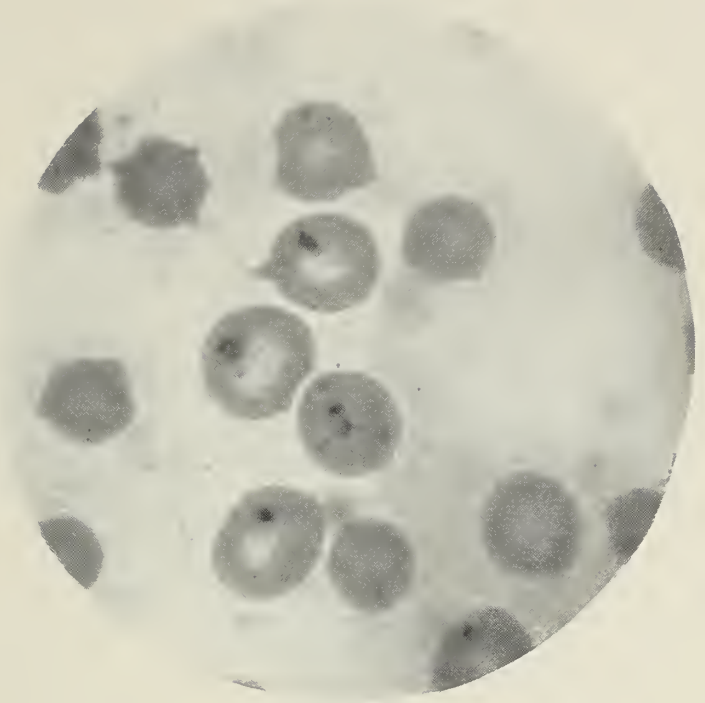

FIG. 4.

Fig. 4. Cover-glass smear of systemic blood of Sheep 14; five corpuscles are infected with Piroplasma ovis. Corpuscle near center of the field has a parasite of stellate iorm. Carbolished polychrome methylen-blue. Photo-micrograph. X 1500.

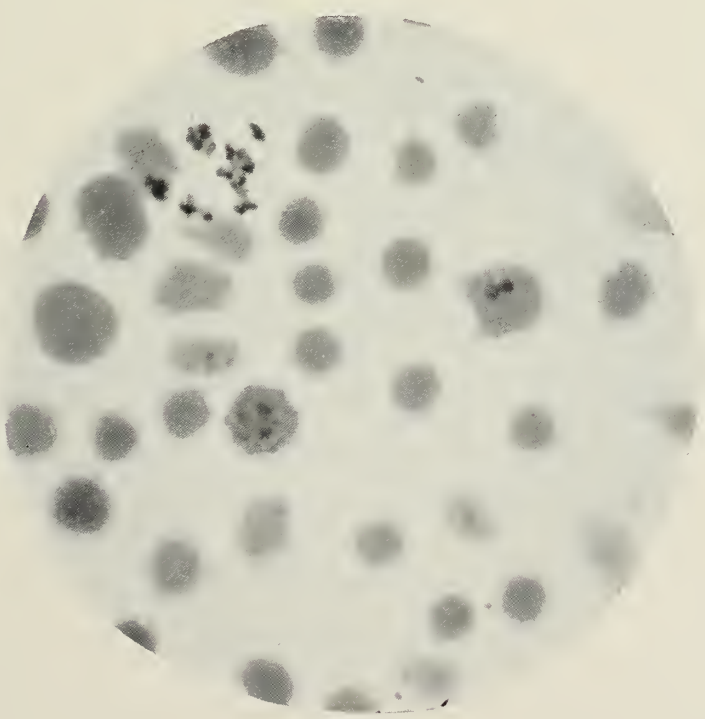

FIG. 5.

Fig. 5. Blood smear (sytemic) of Sheep 13, showing marked piokilocytosis. Two corpuscles with basiphilic granules, and one infected with P. ovis in the binary form. A mass of blood-plateletes to the left. Wright's blood stain. Photo-micrograph. X 1500.

first indication of the onset of the disease. As previously stated, the results were entirely negative. Although there is no conclusive evidence that any multiplication of the parasites occurred after injection, they certainly remained in the circulating blood of the animal for weeks after the operation, as shown in blood smears takens from the ear.

## Conclusions.

The results of the investigation indicate that ictero-haematuria prevails only where conditions are favorable. Just what these favorable conditions are is not clearly understood because of our ignorance of the intermediate host that transmits the disease from sheep to sheep. That the disease is not contagious in the proper sense of the term is sufficiently clear. It is not a question of direct transmission from sheep to sheep, but evidently the germ must pass through at least one and possibly two intermediate hosts. Reasoning from the analogy of closely related germs which produce the malarial fevers of man and birds, Texas cattle fever, etc., this germ undergoes in the intermediate host certain developmental stages absolutely essential to its further propagation. It is obvious that no means of prevention other than purely empirical ones can be adopted until this intermediate host or hosts are known. At present the best course for the sheep-raiser to pursue is to give his flocks the best of care. Experience shows that nourishing and bountiful feed, pure water, and clean, dry pasture are the best prophylactic measures that can be adopted.

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